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Direct Steam Distillation as an Alternative to the Illinois Soil Nitrogen Test

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Development of the Illinois Soil N Test (ISNT) has rejuvenated the search for a soil-based N test to measure potentially mineralizable soil N. Accurate quantification of amino sugar N has been achieved using the ISNT, but issues concerning sample variability and analysis time have led to the discovery of a 10 mol L^{-1} NaOH direct steam distillation (DSD) procedure. Our primary objective was to determine if DSD could be used as a reliable alternative to the ISNT. Laboratory experiments were conducted to compare the two methods based on recovery of N from pure organic compounds, specificity tests to determine amine group hydrolysis, and recovery of ^{15}N-labeled glucosamine N added to soils. Both methods recovered appreciable amounts of amino sugar N from pure compounds and the ISNT had a higher recovery of N from all amino sugar compounds. Recovery of N from glutamine and asparagine was higher using DSD. Direct ^{15}N techniques for recovery of glucosamine N added to six soils showed no significant difference between the two methods within a soil, but resulted in significant differences among soils. Glucosamine-^{15}N recovery significantly and positively correlated with soil total N. Although the ISNT and DSD measure different amounts of amino sugar N and transition amino acid N, they recover relatively the same amount of hydrolyzable N for a given soil, indicating that differences between the methods may not be that significant as both appear to quantify a pool of potentially mineralizable N. Direct steam distillation appears to be a viable alternative to the ISNT in correlation and calibration of crop response for N-fertilizer recommendations due to the short analysis time per sample (~6 min) and the accurate estimation of potentially mineralizable N.

Abbreviations: DSD, direct steam distillation; DT, difference technique; ISNT, Illinois Soil Nitrogen Test.

The quantification of potentially mineralizable N has been a goal of soil testing for nearly half a century. Soil scientists have a basic understanding of the intrinsic and dynamic processes that control N availability in crop production systems, but accurately predicting the mineralization of native soil N on a consistent basis has eluded soil fertility professionals. Organic N is the most prevalent form of N in the soil and can account for as much as 99% of the total N at any given time (Stevenson and Cole, 1999). A wide array of organic N compounds exist in the soil and are most often classified using acid hydrolysis (Stevenson, 1982). Amino acid N comprises the largest portion of the organic N pool and represents about 50% of the total N in a soil, but the amino sugar N is a fraction that represents a more labile N pool (Mengel, 1996; Stevenson, 1996; Stevenson and Cole, 1999). Identification and quantification of a specific fraction of organic N that contributes to the plant-available N will be an essential component in the success of a soil-based N test for fertilizer recommendations.

Mulvaney and Khan (2001) began to research the benefits of diffusion to facilitate fractionation of organic N in soil hydrolysates as an alternative to steam distillation and reported that diffusion was more accurate and less variable. Further work by Mulvaney et al. (2001) led to the use of an improved fractionation technique to quantify amino sugar N, which was then correlated with check plot yield and response to fertilizer N by corn (*Zea mays* L.). Following this discovery, Khan et al. (2001) developed a simple direct soil alkali diffusion technique that could accurately quantify amino sugar N in soils. This alkali diffusion (2 mol L^{-1} NaOH) provides a quick and easy alternative to quantify amino sugar N rather than the lengthy acid hydrolysis method and was named the Illinois Soil N Test (ISNT). Results of the ISNT were highly correlated with hydrolyzable amino sugar N and specificity tests were performed using ^{15}N-labeled glucosamine that validated the test's ability to recover amino sugar N. Recovery of ^{15}N using ISNT was quantitative for glucosamine and the soil test was able to accurately classify the responsiveness of corn to N fertilization on 25 Illinois soils (Khan et al., 2001). Several other researchers have shown the versatility of the ISNT based on significant correlations with aerobic and anaerobic incubations as well as acid hydrolysis (Bushong et al., 2007; Sharifi et al., 2007). The implementation of the ISNT into mainstream soil testing has been met with much criticism due to issues of variability and analysis time. Several modifications to the original ISNT were made by the developers and included electronic controllers and

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sample rotation, while other researchers suggested the use of enclosed griddles and incubators (Khan et al., 2001; Klapwyk and Ketterings, 2005). The initial success of the ISNT was offset by issues concerning the reproducibility of results, high sample variability, and analysis time.

The relatively short analysis time of steam distillation makes it a popular analytical technique and Bushong et al. (2008) investigated the use of a DSD technique as an alternative to ISNT for the quantification of amino sugar N. The results of DSD techniques have been highly correlated with ISNT results using a wide range of NaOH molarities and procedural techniques (Sharifi et al., 2007; Bushong et al., 2008). Although DSD techniques have been highly correlated with the ISNT, the higher recovery of total hydrolyzable N from the soil by DSD suggests that it may be measuring more labile forms of organic N (Bushong et al., 2008). Utilizing the difference technique (DT), Bushong et al. (2008) reported a significant difference in the recovery of glucosamine N added to the soil due to method (ISNT or DSD) and soil texture. These findings suggest that the ISNT and DSD methods may be measuring different types of soil organic N. This theory is supported by discrepancies in the following observations: (i) the significant correlation of the two methods in quantifying potentially mineralizable soil N, and (ii) significant differences by method in glucosamine N recovery from soils of varying texture. Utilization of a ^{15}N isotopic technique, as presented in Khan et al. (2001), may provide a more reliable way to compare the recovery of glucosamine N from soil by the ISNT and DSD.

Direct steam distillation has shown potential in replacing the ISNT as a predictor of potentially mineralizable N due to much shorter analysis times, but has raised concern over the difference in recovery of glucosamine N from the soil compared with the ISNT. Before the inclusion of DSD into mainstream soil testing as a timely and accurate alternative to the ISNT, a detailed comparison of the two methods across a series of recovery and specificity tests is needed. We hypothesized that ISNT and DSD were measuring similar organic N compounds and analysis by either method would yield similar results. Therefore, a series of objectives were developed to evaluate the compatibility of these two methods: (i) evaluate the ability of ISNT and DSD to recover pure organic N compounds, (ii) evaluate the recovery of specific amine groups from ^{15}N-labeled pure organic N compounds using the ISNT and DSD, and (iii) utilize ^{15}N direct and difference techniques to compare the recovery of glucosamine N from the soil by the ISNT and DSD.

MATERIALS AND METHODS

Soil Samples

Surface soil samples (0–15 cm) were collected from six soil series across the southern U.S. Great Plains, with four being from Arkansas, one from Oklahoma, and one from Texas to represent a wide range in soil texture and total N (Table 1). Two soils were selected to represent each of the three predominant soil particle sizes: sand, silt, and clay. Soils were collected from areas of agricultural production and represented a number of cropping systems whose species included rice (*Oryza sativa* L.), soybean [*Glycine max* (L.) Merr.], peanut (*Arachis hypogeal* L.), and cotton (*Gossypium hirsutum* L.). Before analysis, the soil was air dried and ground to pass through a 2-mm sieve. Particle size analysis was performed using a 24-h hydrometer method according to Craze et al. (2003). Organic C and total N were determined using dry combustion techniques (Nelson and Sommers, 1996) and inorganic N (NH_4^+–N and NO_3^-–N) was based on a salicylate colorimetric technique outlined by Mulvaney (1996). Soil pH was measured with a glass electrode in a 1:2 (w/v) soil/water mixture.

Pure Compounds

Eighteen reagent-grade samples were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO), 17 of which were organic N compounds and the other [$(NH_4)_2SO_4$] served as a check sample (Table 2). Ammonium sulfate was chosen as the method check since recovery of NH_4–N using both DSD and ISNT should be ~100%. Each compound was classified into one of five groups (i.e., amino acid, amino sugar, nucleic acid, transition, or miscellaneous) based on structure or metabolic significance and the proportion of N (6–52%) varied greatly among the compounds. Before use, the purified N compounds were dried over anhydrous $CaSO_4$ for a minimum of 72 h and aqueous solutions for each compound were prepared containing 1 mg N mL^{-1} by dissolution in 50 mL of deionized water. Aqueous solutions containing the purified N compounds were used within 24 h after preparation and stored in a refrigerator (<10°C) until analysis. Asparagine and glucosamine labeled with 99 atom% ^{15}N (Isotec, Miamisburg, OH) were used for a specificity test as well as recovery from the soil. Two amine groups can be found on asparagine and for this reason only a single amine group (Fig. 1) was labeled with ^{15}N to identify whether multiple amine groups are being hydrolyzed equally. Each of the ^{15}N-labeled compounds were isotopically diluted with pure reagent-grade unlabeled material and used to make an aqueous solution containing 200 mg N L^{-1} at ~1.5 and ~1.2 atom% ^{15}N

Table 1. Site and chemical characterization of the six soils utilized in the ^{15}N-glucosamine-N recovery experiment.†

Soil series	State	Crop	Taxonomy	Texture	Clay	Sand	pH‡	Organic C§	Total N§	NH_4–N¶	NO_3–N¶
					—%—			—g kg^{-1}—		—mg kg^{-1}—	
Ganado	Texas	rice	Hapludert	sandy loam	13	70	6.5	7.8	0.39	9.2	1.26
Pond Creek	Oklahoma	peanut	Argiustoll	loamy sand	6	86	6.5	6.6	0.35	5.4	0.52
Dewitt	Arkansas	soybean	Albaqualf	silt loam	14	17	6.1	11.7	0.98	10.0	0.31
Henry	Arkansas	soybean	Fragiaqualf	silt loam	17	8	6.2	12.3	1.45	13.7	0.50
Portland	Arkansas	cotton	Epiaquert	clay loam	39	21	7.2	7.9	1.13	10.9	0.63
Perry	Arkansas	rice	Epiaquert	clay	68	5	7.2	10.8	1.62	8.7	0.16

† All analytical data are reported as the mean of three replicate determinations.

‡ 1:2 soil/water ratio.

§ Determined by dry combustion techniques (Nelson and Sommers, 1996).

¶ Determined by salicylate colorimetric techniques (Mulvaney, 1996).

Table 2. Description of N-based compounds used in the experiment and a comparison of the Illinois Soil N Test (ISNT) and direct steam distillation (DSD) for recovery of N in selected pure compounds.

Compound	Classification	N content	Recovery of N				Statistical significance
			ISNT		DSD		
			Mean	SD	Mean	SD	
			%				
Arginine	amino acid	32.2	<5	0.79	<5	0.37	NS
Glycine	amino acid	18.6	<1	0.05	<1	0.12	***
Lysine	amino acid	19.2	<1	0.35	<1	0.38	†
Proline	amino acid	12.2	<1	0.11	<5	0.34	***
Tyrosine	amino acid	7.73	<1	0.32	<5	0.34	*
Tryptophan	amino acid	13.7	<1	0.20	<1	0.41	†
Galactosamine	amino sugar	7.81	85	1.06	75	2.61	***
Glucosamine	amino sugar	7.81	93	0.66	83	3.75	***
Mannosamine	amino sugar	6.49	92	1.30	82	1.24	***
N-acetyl-glucosamine	amino sugar	6.33	60	2.45	38	0.93	***
Adenine	nucleic acid	51.7	<1	0.16	<1	0.13	*
Guanine	nucleic acid	46.3	<1	0.30	<1	0.13	NS
Cytosine	nucleic acid	37.8	<1	0.30	<1	0.23	*
Uracil	nucleic acid	25.0	<1	0.10	<1	0.58	***
Asparagine	transition	21.2	49	1.15	58	1.67	***
Glutamine	transition	19.2	25	3.05	48	2.03	***
Urea	miscellaneous	46.6	<5	0.32	11	1.85	***
$(NH_4)_2SO_4$	check	21.2	96	0.84	99	0.38	***

* Significant at the 0.05 level.

*** Significant at the 0.001 level.

† Significant at the 0.1 level; NS, no statistical significance.

for glucosamine and asparagine, respectively. This concentration of N was chosen as it represents a reasonable recovery of N using the ISNT on these particular soils (Ross, 2007). The exact enrichment of each ^{15}N-labeled solution was determined experimentally using semimicro-Kjeldahl steam distillation techniques (Bremner, 1996) and analyzed for atom% ^{15}N. Isotope analysis for the recovery and specificity tests was conducted at the University of Illinois on a Nuclide/MAAS 3–60-RMS double collector mass spectrometer (Nuclide Corp., Bellefont, PA) using an automated Rittenburg system (Mulvaney et al., 1990).

Illinois Soil Nitrogen Test

Each N compound was analyzed using the accelerated diffusion method and chamber developed by Mulvaney and Khan (2001) and Khan et al. (2001). Each analyte was placed in a diffusion chamber and combined with 10 mL of 2 mol L^{-1} NaOH. Within each modified lid, a petri dish was placed containing 5 mL of a 4% H_3BO_3 indicator solution. Diffusion chambers were immediately placed on preheated hot plates modified to maintain a temperature of 48 to 50°C. Samples were rotated at 1.5 and 3 h, then removed after 5 h of heating. Upon removal from the hot plate, the diffusion chamber was opened and the petri dish was released from the lid. The H_3BO_3 indicator solution was diluted with 5 mL of deionized water and titrated to an established endpoint using an automatic titrator to determine NH_4–N. Samples analyzed for atom% ^{15}N were titrated and then treated according to the procedure outlined by Khan et al. (2001), where the H_3BO_3 was removed using methanol and the $(NH_4)_2SO_4$ was solvated using deionized water and prepared for ^{15}N analysis.

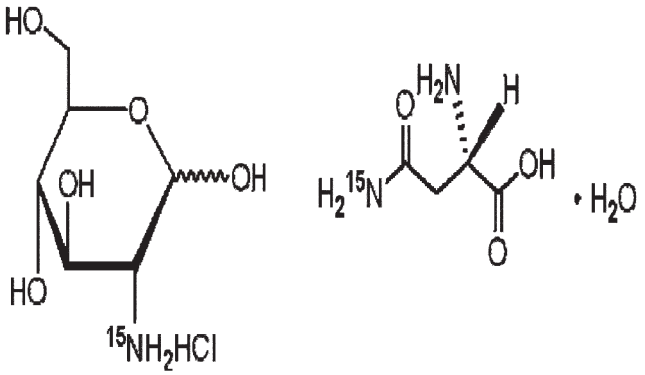

Fig. 1. Nitrogen-15-labeled glucosamine (left) and asparagine (right) used during the experiment. Note the single labeled amine group on asparagine.

Direct Steam Distillation

A modified steam distillation technique was used based on the work by Bushong et al. (2008). Each analyte was placed directly in a Kjeldahl flask with the flask attached directly to the still, and 10 mL of 10 mol L^{-1} NaOH was added to the flask via the addition cup located on top of the apparatus. Steam distillation was conducted at a rate of 7 mL min^{-1} until 35 mL of distillate was collected in 5 mL of 4% H_3BO_3 indicator solution. The amount of NH_4–N captured in the distillate was quantified using acidimetric titration techniques to a predetermined endpoint. The duplicate aliquot technique was performed on all samples containing ^{15}N to minimize the cross-contamination error described by Mulvaney (1986). That is, the first distillation conditioned the still and was caught in H_3BO_3–indicator solution for quantification of NH_4–N and the second distillation was caught in 0.1 mol L^{-1} H_2SO_4 and used for ^{15}N analysis.

Recovery of Pure Compounds

The recovery percentage of N for each compound was determined with the DSD and ISNT methods using a completely randomized design with four replications. One milliliter of each aqueous solution containing 1 g N L^{-1} was analyzed by each method and the N recovery determined.

Nitrogen recovery curves were developed for asparagine N, glucosamine N, and *N*-acetyl-glucosamine N by each method. An aqueous stock solution containing 2 mg N mL^{-1} of each compound was prepared and used to create 10 rates of N for each compound ranging from 0.2 to 2 mg N mL^{-1}. The experiments were set up in a completely randomized design with three replications. Replicate data were analyzed by regressing the milligrams of N recovered vs. the milligrams of N added. Slopes within compounds were used to compare the recoveries of the two methods and comparison within a method was used to determine the differences in compound recovery.

Specificity tests for each method were conducted using the aqueous solutions containing ^{15}N-labeled asparagine (1.18 atom% ^{15}N) and glucosamine (1.54 atom% ^{15}N). Compounds were analyzed for NH_4–N and ^{15}N using the ISNT and DSD procedures described

above. Comparison of the known atom% ^{15}N of the glucosamine solution to the atom% ^{15}N glucosamine N hydrolyzed by the DSD and ISNT was used to determine the accuracy of each method. The same comparison for asparagine was used to identify the degree of hydrolysis for each of the two amine groups by DSD and ISNT (Fig. 1). The recovery percentage of the labeled N (R) was calculated based on a modification of the equation presented in Mulvaney and Khan (2001):

$$R = M(T - U)/X(L - U) \quad [1]$$

where M represents the mass (μg) of N recovered during analysis and X represents the micrograms of N added. The remaining variables represent the measured atom% ^{15}N values for the treated sample (T), the untreated sample (U), and the labeled N added (L) (Mulvaney and Khan, 2001).

Recovery of Nitrogen-15-Labeled Glucosamine from Soil

Six soils (Table 1) were selected to investigate the effects of soil texture on glucosamine N recovery by DSD and the ISNT using the difference and ^{15}N direct methods. Experiments were set up in a completely randomized design with three replications. An aqueous solution of ^{15}N-labeled glucosamine (1.54 atom% ^{15}N) was prepared as described above. One-gram soil samples were treated with 0 or 400 μg N of the ^{15}N-labeled glucosamine, briefly mixed, and were subjected to analysis within 30 min. For the DSD method, sequential distillations were performed as described above. The recovery percentage using the ^{15}N isotopic technique was determined using Eq. [1] and the recovery percentage by the DT was calculated based on the following equation:

$$R = (M - S)/X \quad [2]$$

where S represents the mean micrograms of NH_4–N recovered from the soil for each method based on four replicates.

Statistical Analysis

The recovery of pure compounds experiment was a completely randomized design with treatments arranged in a split-plot structure, with method representing the main-plot factor and product representing the subplot factor. Treatments were replicated four times. Analysis of variance was used to compare mean recovery for ISNT and DSD by classification (e.g., amino acid or amino sugar), with significant differences interpreted at the $\alpha = 0.05$ level.

Simple linear regression techniques were used to compare the recovery of glucosamine, asparagine, and *N*-acetyl-glucosamine by the DSD and ISNT methods. The fit model function was used to identify differences in slope and intercept coefficient values for a given compound between methods at the $\alpha = 0.05$ level.

Specificity tests were analyzed as a split-plot treatment structure, with method representing the main-plot factor and product representing the subplot factor in a completely randomized design with three replications. Student's *t*-tests were used to separate means using LSD(0.05).

Recovery of ^{15}N-labeled glucosamine from the soil was analyzed as a split-plot treatment structure, with method representing the main-plot factor and soil representing the subplot factor in a completely randomized design with three replications. Analysis of variance was conducted on data from the difference and ^{15}N direct technique separately and Student's *t*-test was used to separate means using Fisher's protected LSD method, with significant differences interpreted when $P < 0.05$. All statistical analyses were performed using JMP 7 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

To promote sustainable crop production, more accurate techniques to predict crop N fertilizer requirements will have to be developed. In the past, several soil-based chemical and biological methods were developed and investigated, but no one test gained wide acceptance for N fertilizer recommendations (Stanford, 1982). Until recently, the majority of crops have relied solely on inorganic N concentrations in the soil to adjust or predict crop N needs (Bundy and Meisinger, 1994) or crop response trials (e.g., variety × N rate), but these methods do not accurately predict the amount of potentially mineralizable N. The development of the ISNT sparked a new wave of research into a soil-based analytical method focusing on amino sugar N as a specific fraction of labile organic N that could be correlated with crop response parameters such as the economically optimum N rate, response to N fertilization, and delta yield in corn (Khan et al., 2001; Mulvaney et al., 2001, 2006; Klapwyk and Ketterings, 2006; Williams et al., 2007a,b). Acceptance of the ISNT has been due to the simplicity of the methodology in terms of equipment and protocol, but issues of sample time and variability have led to the search for alternative methods (Klapwyk and Ketterings, 2005; Sharifi et al., 2007; Williams et al., 2007a,b; Bushong et al., 2008). Direct steam distillation techniques have shown the most promise as viable alternatives to the ISNT based on data presented by Bushong et al. (2008), where significant correlations were found with DSD and the ISNT as well as anaerobic incubation. Although DSD and the ISNT have correlated well, the slope and intercept values suggest that more hydrolyzable N is recovered using DSD (Bushong et al., 2008). Before the implementation of DSD as an alternative to the ISNT, it is important to understand if the two methods are similar in the types of compounds they hydrolyze as well as the amount of N recovered.

Comparison of Methods for Hydrolyzable-Nitrogen Recovery from Pure Compounds

Pure organic N compounds of varying structure and N content were analyzed using the ISNT and DSD. The 18 compounds were categorized based on structure or metabolic significance and the N content of each is presented in Table 2. Ammonium sulfate was used as a check, as near 100% recovery of NH_4–N by both methods should be expected. Recovery of $(NH_4)_2SO_4$ was comparable for both methods and >96% for each (Table 2). Amino acid N was recovered in trace amounts by both methods (<5%), but was significantly different for all amino acids except arginine. Recovery of amino sugar N was significantly different at an $\alpha = 0.001$ level for all amino sugar compounds, with the ISNT recovering as much as 22% more N than DSD (Table 2). Although recovery of nucleic acids was <1% for all compounds, there was a significant difference between methods for every compound except guanine (Table 2). Glutamine and asparagine were classified as transition amino acids due to the differences in structure and function from the other amino acids used in the study. The ISNT and DSD recovered significantly different amounts ($\alpha = 0.01$) of these two

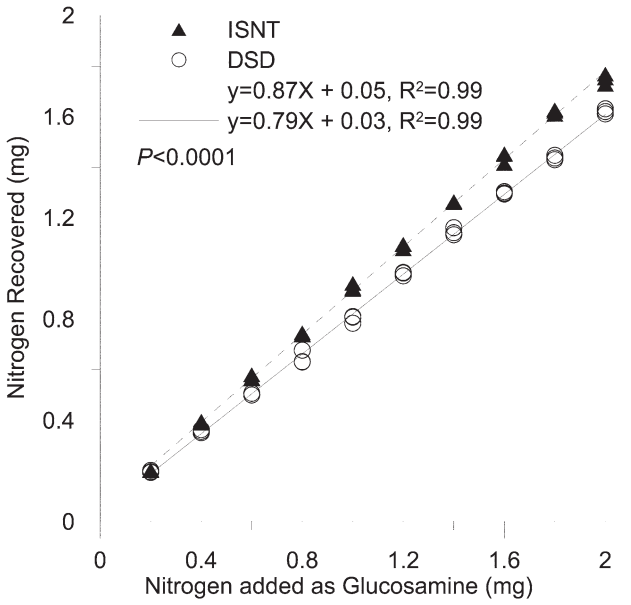

Fig. 2. Recovery of glucosamine N by the Illinois Soil N Test (ISNT) and direct steam distillation (DSD). Standard error for the slope and intercept term were 0.0035 and 0.0044, respectively, at the α = 0.05 level.

compounds. The ISNT recovered 49 and 25% and the DSD recovered 58 and 48% for glutamine and asparagine, respectively. Urea N recovery was more than two times greater for DSD than for the ISNT, which is significant at the α = 0.01 level.

For all compounds other than arginine and guanine, the results suggest that there are significant differences between the ISNT and DSD in the recovery of hydrolyzable N from pure organic N compounds (Table 2). Amino sugars and the transition compounds resulted in >25% recovery and could be significant contributors to the labile soil N pool. Glucosamine N recovery by the ISNT was similar to the results of Khan et al. (2001), validating the test results for amino sugar N recovery. The ISNT resulted in higher N recovery than DSD by at least 10% from all amino sugars analyzed. Although DSD recovered a significantly lower amount of amino sugar N, the method was still able to hydrolyze as much as 83% of the glucosamine N (Table 2). Direct steam distillation resulted in a significantly higher recovery of glutamine N and asparagine N than the ISNT, which could offset the significantly lower recovery of amino sugar N and account for the similarities in total hydrolyzable N recovery from the soil samples analyzed by each method.

Nitrogen Recovery Curves for Glucosamine, Asparagine, and *N*-Acetyl-Glucosamine

To better understand the relationship between the types of compounds being analyzed and the methods involved, recovery curves were developed for glucosamine N, asparagine N, and *N*-acetyl-glucosamine N across a range of rates from 0.2 to 2 mg N. Comparison of the ISNT and DSD methods for recovery of glucosamine N resulted in significantly different slopes (P < 0.0001), with ISNT having a greater slope and intercept value (Fig. 2). The amino sugar with the least amount of N recovered by the two methods was *N*-acetyl-glucosamine (Fig. 3). There was a significant difference in the slopes (P < 0.0001) for each method, with the ISNT having a slope that was nearly twice as great as DSD. Recovery of asparagine by the two methods was also investigated due to the difference in structure compared with the other compounds and moderate rates of N recovery by DSD and ISNT (Table 2). The recovery curve for asparagine N with DSD (Fig. 4) had a significantly greater slope than the ISNT (P < 0.0001) and the N recovered was comparable numerically to the results presented in Table 2.

The linear relationships and high coefficients of determination (R^2 = 0.99) for the N recovered with both methods suggest that analytical conditions such as analysis time and

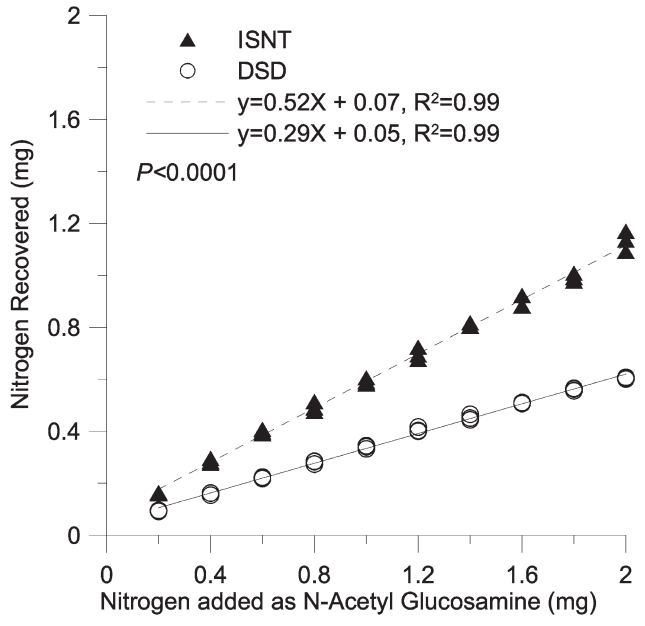

Fig. 3. Recovery of *N*-acetyl-glucosamine by the Illinois Soil N Test (ISNT) and direct steam distillation (DSD). Standard error for the slope and intercept term were 0.0035 and 0.0044, respectively, at the α = 0.05 level.

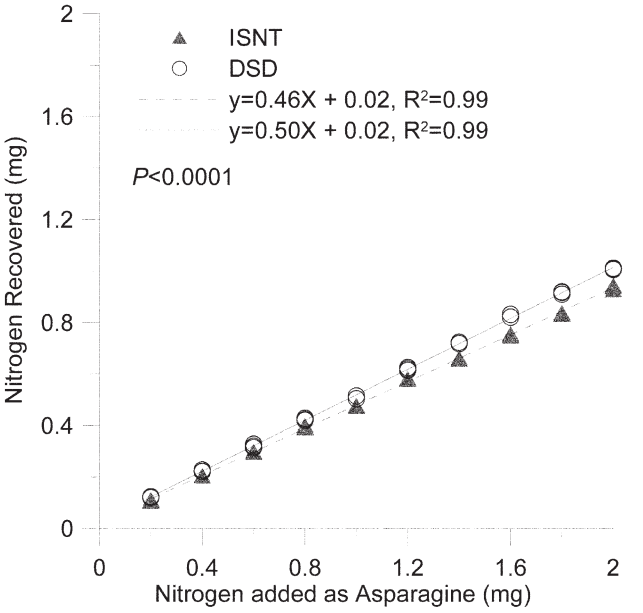

Fig. 4. Recovery of asparagine by the Illinois Soil N Test (ISNT) and direct steam distillation (DSD). Standard error for the slope and intercept term were 0.0014 and 0.0018, respectively, at the α = 0.05 level.

alkaline strength were not limiting within the range of N analyzed. The consistency of both methods in the recovery of a particular compound across several N rates exhibits their versatility for a wide range of soils with different amounts of hydrolyzable N.

Table 3. Recovery of 15N-labeled amine groups with the Illinois Soil N Test (ISNT) and direct steam distillation (DSD) compared with the total N digestion (TN digest).

Compound	Recovery of ^{15}N-labeled amine groups			
	TN digest†	ISNT	DSD	LSD(0.05)
	atom% ^{15}N			
Glucosamine	1.54	1.55	1.54	0.03
Asparagine	1.18	1.82	1.83	0.05

† Determined by the Kjeldahl method (Bremner, 1996).

Specificity Tests for Nitrogen-15-Labeled Glucosamine and Asparagine

The ^{15}N isotope direct technique was used to compare the ISNT and DSD for recovery of N from specific amine groups on glucosamine and asparagine. Analysis of ^{15}N-labeled glucosamine by total N digestion, ISNT, and DSD resulted in no significant differences among the three methods at the $\alpha = 0.01$ level (Table 3). Specificity tests for glucosamine resulted in no significant differences between the ISNT, DSD, and total N digestion, as expected, and were similar to the results presented by Khan et al. (2001).

The amide group on asparagine was labeled to determine if the two amine groups were hydrolyzed equally by the ISNT and DSD (Fig. 1). The total N digest serves as the baseline and gave the exact atom% ^{15}N (1.18 atom% ^{15}N) of the labeled asparagine compound since both amine groups are being measured in equal proportions (Table 3). The atom% ^{15}N of the N recovered by the ISNT and DSD were not significantly different from one another, but both were greater than the N recovered with the total N digestion. These results indicate that the ISNT and DSD recovered a significantly greater amount of the ^{15}N-labeled amide group than the unlabeled amine group on asparagine.

The data for asparagine provide valuable insight into the types of organic N compounds that are being hydrolyzed from soil organic matter. Both the ISNT and DSD recovered >90% of the ^{15}N-labeled amide group on asparagine, suggesting that these methods are preferentially hydrolyzing certain amine groups. Alkaline hydrolysis appears to quantify the amide- or carbonyl-associated N, whereas acid hydrolysis is effective at recovering the amine N associated with the R group. Glutamine has many of the same chemical characteristics as asparagine and represents an important component of the amino acid pool in soils. The recovery of asparagine by the ISNT and DSD indicate that amide-associated N should be a readily mineralizable form of organic N. Understanding the types of compounds and the relative bond strengths that can be hydrolyzed using either method will allow researchers to identify which types of compounds are making significant contributions to the labile and potentially mineralizable N pools.

Recovery of Nitrogen-15-Labeled Glucosamine from the Soil

The site and chemical characteristics of the six soils used in the experiment are presented in Table 1. These six soils represent a wide range of soil textures and geographic regions, but were all sampled from soils cultivated for various types of crop production. The clay content ranged from 6 to 68% (Table 1) and total N ranged from 0.35 to 1.62 g kg^{-1} (Table 1) among the six soils. Glucosamine N recovery from each soil with the ISNT and DSD was determined using both the difference and ^{15}N direct techniques based on either Eq. [1] or [2]. Using the DT approach, the recovery of glucosamine N by the ISNT and DSD methods within a given soil was significantly different (Table 4). A comparison of soils within each method resulted in no significant differences. The results of the DT approach were similar to the data presented by Bushong et al. (2008), where the ISNT resulted in a significantly higher recovery of glucosamine N than DSD for all soils except the Portland. Quite different recoveries of glucosamine N were measured with the ISNT and DSD in most soils, however, when the ^{15}N-isotope direct technique was used (Table 4). The ^{15}N-isotope direct technique revealed that there was no significant difference in the recovery of the added ^{15}N-labeled glucosamine N by the ISNT and DSD methods for a given soil (Table 4). In contrast, there was a significant difference within a method among soils when using the ^{15}N-isotope technique. The recovery percentage of added ^{15}N-labeled glucosamine N regressed against soil total N showed a significant, negative correlation (Fig. 5). In addition, a significant, positive correlation was obtained when the recovery percentage of ^{15}N-labeled glucosamine N was regressed vs. the sand content for each soil (Fig. 6).

Our results identify some of the problems associated with using the DT rather than a ^{15}N direct isotopic approach. Glucosamine N recovery between the ISNT and DSD using the ^{15}N technique was not different for a particular soil, but did result in significant differences between soils as soil characteristics changed (Table 4). Utilizing the results of the ^{15}N direct technique and the relationship with soil total N, it is apparent that soil total N plays a role in the recovery of glucosamine N with the ISNT and DSD (Fig. 5). As soil total N increased, the recovery of added ^{15}N-glucosamine N decreased, but the recovery of hydrolyzable N remained relatively constant (based on DT results). These results suggest that as the total N of the soil increases, the amount of hydrolyzable N also increases and the recovery of an added N source will be

Table 4. Recovery of glucosamine N with the Illinois Soil N Test (ISNT) and direct steam distillation (DSD) methods using difference and ^{15}N isotopic techniques in six soils of varying texture.

Soil	Glucosamine N recovery			
	Difference		^{15}N Analysis	
	ISNT	DSD	ISNT	DSD
	%			
Ganado	99	91	84	85
Pond Creek	98	87	88	89
Dewitt	100	86	76	78
Henry	95	88	67	68
Portland	93	93	75	76
Perry	98	91	67	66
LSD(0.05) within a soil	2.7		7.2	
LSD(0.05) among soils	13.1		2.2	

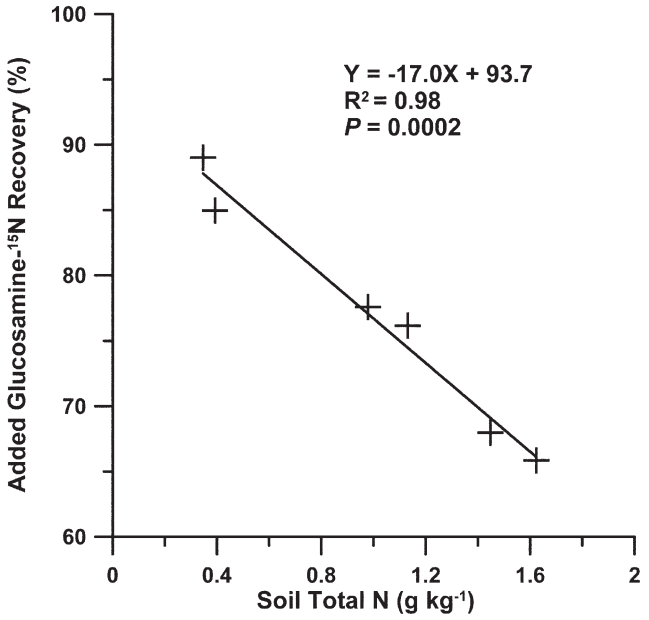

Fig. 5. Linear regression of the ^{15}N-labeled glucosamine N recovery vs. soil total N. Standard error for the slope and intercept term were 1.35 and 1.48, respectively at the α = 0.05 level.

reduced proportionally. The positive correlation between glucosamine N recovery and sand content could be an artifact of the relationship between soil texture and soil total N, but would suggest that as sand content increases, the proportion of hydrolyzable N in the soil decreases, resulting in a higher recovery of an added N source. Recovery of glucosamine N added to the soil using the results of the DT highlights key differences in the chemistry and time requirements of the two methods. The moderately alkaline ISNT appears to recover more amino sugar N, whereas the strongly alkaline DSD appears to recover more total hydrolyzable N due to differences in alkaline concentration, analysis time, and temperature.

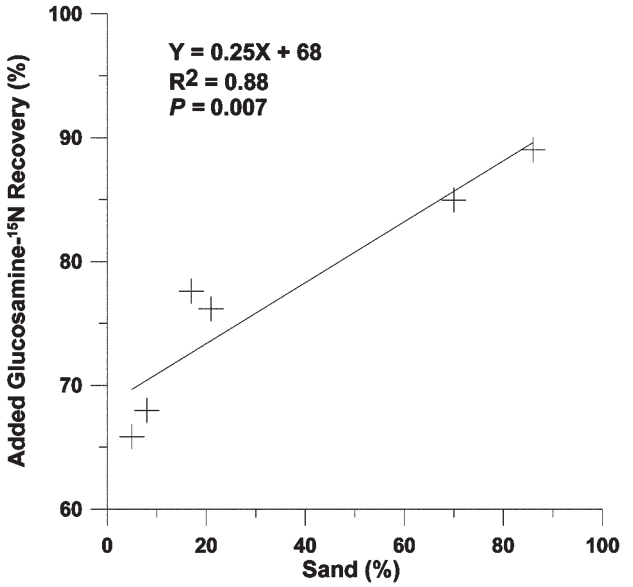

Fig. 6. Linear regression of the ^{15}N-labeled glucosamine N recovery vs. the soil sand content. Standard error for the slope and intercept term were 0.05 and 2.24, respectively at the α = 0.05 level.

CONCLUSIONS

Our results highlight the similarities and differences between the ISNT and DSD techniques in a series of laboratory experiments. Recovery of amino sugar N was significantly higher for the ISNT, but DSD resulted in higher amounts of N recovered from transition amino acids, which can represent a significant portion of soil organic N and explain the similarity of the ISNT and DSD when soil hydrolyzable N is compared. Higher recovery of amino sugar N using the ISNT may be due to the lengthy diffusion time (~5 h) compared with the relatively short distillation time of DSD (<6 min). Specificity tests using ^{15}N-labeled asparagine resulted in an equal recovery of N, but identified a preference for the amide- or carbonyl-associated group on the asparagine compound. In the future, identification of bond strengths for specific amine groups may lead to a better understanding of the hydrolyzable-N fraction and the development of a more precise soil-based N test for N fertilizer recommendations.

Using the ^{15}N direct isotopic approach, it was clear that soil total N plays an important role in the recovery of glucosamine N added to the soil. An increase in total soil N lowered the recovery of N that was added to the system but did not affect the hydrolyzable N measured. An increase in the hydrolyzable N as soil total N increases suggests that both of these methods measure a relatively constant fraction of soil organic N and that the amount of hydrolyzable N or potentially mineralizable N increases proportionally. Although the ISNT and DSD measure different amounts of amino sugar N and transition amino acid N, they result in relatively the same amount of hydrolyzable N for a given soil. Differences in glucosamine N recovery from the soil by each method may be accounted for in their quantification of hydrolyzable NH_4^+, which is hard to identify as this fraction can include exchangeable NH_4^+, some amino sugar N, and other labile N sources. Differences between the ISNT and DSD may not be that significant, as it appears that they both quantify a pool of potentially mineralizable N and can be used successfully to predict crop N response parameters. Direct steam distillation is a viable alternative to the ISNT due to a short analysis time and high level of accuracy, two important factors when considering the selection of a soil test method.

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